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Genetics

Location of the genes controlling H-Y antigen expression and testis determination on the mouse Y chromosome

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ABSTRACT **Sex-reversed XX male mice that carry the variant form of the testis-determining Sxr region, Sxr', do not express male-specific H-Y antigen. In a stock of mice segregating for Sxr', we detected an exceptional XX male that proved positive for H-Y antigen. DNA fingerprinting revealed that the banding pattern characteristic of Sxr' had been replaced by the pattern associated with the native testis-determining region of the normal Y chromosome of that stock, presumably by pairing and crossing-over between the two testis-determining regions of the father's Y Sxr' chromosome. Pairing between the two ends of such a chromosome in a loop-like configuration has been observed by electron microscopy. However, an anomalous crossing-over event of this kind would only give rise to the observed result if the native homologue of the Sxr region were situated on the very minute short arm of the Y chromosome. We therefore conclude that the two linked genes *Tdy* and *Hya*, controlling testis determination and H-Y antigen expression, respectively, are located on the short arm of the mouse Y chromosome.**

Male mice carrying Sxr (sex reversed) [Tp(Y)1Ct] attached to the pseudoautosomal region of their Y chromosome transmit the extra testis-determining Sxr region to 50% of their X/Y and 50% of their X/X progeny (1). The X/Y Sxr individuals are carrier males, like their father; the X/X Sxr individuals are sex-reversed X/X males. Since X/X Sxr males are positive for the male-specific antigen H-Y, the expression of this antigen must be controlled by the Sxr region (2). A variant region has been described (3), termed Sxr', which still carries the genetic information responsible for testis determination (*Tdy*), but which has lost *Hya*, the gene responsible for the expression of H-Y antigen. Unexpectedly, an X/X male fathered by an X/Y Sxr' male proved to be positive for H-Y antigen. We show here that this male can be explained as the product of meiotic pairing and recombination between the two testis-determining regions of the father's Sxr'-carrying Y chromosome, provided that the region containing *Hya* and *Tdy* in the normal mouse Y chromosome is located on the short arm, and not (as has been widely assumed) on the proximal part of the long arm.

MATERIALS AND METHODS

The Sxr' variant (3) was first detected in a female heterozygous for the X 1-1 autosome translocation *T(X;16)16H*, mated to a male hemizygous for the X chromosome-linked marker tabby. The tabby stock, which thus provided the Y chromosome for later X/Y Sxr' males, came from the Institute of Animal Genetics (Edinburgh). X/Y Sxr and X/Y Sxr' male mice have now been back-crossed repeatedly to C57BL/6Mcl females; both stocks (termed CB and CB', respectively) were in the 7th–8th back-crossed generations at the time the anomalous male was detected. X/X males in both stocks were identified by their small testis size (15–25 mg), detected by palpation and checked at autopsy. When required, the identification was confirmed by testis histology.

Chromosome preparations were made from blood lymphocyte cultures and G-banded by standard procedures. For *in situ* hybridization to chromosomes, ^{35}S-labeled RNA probe was prepared to a specific activity of 2×10^8 dpm/μg, and hybridization and autoradiography were carried out as detailed in ref. 4. The probe corresponded to the *Pst* I/*Bam*HI fragment of the Bkm-related *Drosophila* DNA clone 2(8) (5) and hybridizes to sequences found predominantly on the Y chromosome in mice (6). For DNA fingerprinting, DNAs were digested with the restriction enzymes *Hin*fI and *Hae* III, gel electrophoresed, and hybridized in the gel with the synthetic oligonucleotide probe $(GATA)_4$ as described in ref. 7.

For H-Y testing, spleen cell suspensions were made from partial splenectomy samples taken under ether anesthesia from mice to be tested for H-Y. All mice were of the *H-2^b* haplotype. The cell suspensions were tested *in vitro* (*i*) for their capacity to stimulate proliferation of H-Y-specific T-cell clones and (*ii*) as target cells for H-Y-specific *H-2^b*-restricted cytotoxic T cells, according to methods detailed previously (3). Briefly, T-cell clone cultures were incubated with stimulator spleen cells; [^{3}H]thymidine was added for the last 6 hr of culture. They were then harvested and counted for β emissions. For cytotoxic T-cell tests, attacker cells were mixed with ^{51}Cr-labeled target cells at ratios of 30:1, 10:1, 3:1, and 1:1. After 3 hr incubation, 100-μl volumes were removed from each well for γ-counting. As controls, target cells were incubated in 5% Triton X-100 for maximum lysis and in medium alone as a medium control. Techniques for *in vivo* immunization with spleen cells, and subsequent skin grafting, are described in ref. 8.

RESULTS AND DISCUSSION

We tested 178 presumed X/X Sxr' males up to March 1988 for H-Y status; all but one proved negative. The exceptional male, no. 719, gave a positive result both on cytotoxicity testing and on an H-Y-specific T-cell clone proliferation assay; on the same tests, three of his X/X brothers proved negative for H-Y antigen (Table 1). A second spleen sample was taken, and the positive result was confirmed. In addition, groups of two to four C57BL/6Mcl females received injections of spleen cells from male no. 719 or from three control CB strain X/X Sxr males. Subsequent grafts of CB strain X/X Sxr male skin to these females showed a mean survival time of 13.0, 13.0, and 15.0 days for the three control groups and 14.3 days for the group immunized with cells from male no. 719. Primary grafts of H-Y-positive skin from CB or CB' strain males onto C57BL/6Mcl females that have not been

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Table 1. H-Y antigen *in vitro* typing data on mouse no. 719 and three of his X/X Sxr′ brothers (nos. 726, 727, and 728)

Strain of origin of stimulator or target spleen cells	Proliferation of clone, cpm*		Cytotoxicity of T cells†		H-Y status
	B9(H-Y + A^b)	10-2(H-Y + D^b)	anti-H-2^b	anti-H-Y^b(H-Y + D^b)	
C57BL/10 (♂)	62,556	24,642	36	54	+
C57BL/10 (♀)	125	530	37	6	−
719 (♂)	96,569	48,878	37	57	+
726 (♂)	196	654	36	5	−
727 (♂)	402	638	56	0	−
728 (♂)	421	845	10	0	−

*T-cell clone cultures were incubated with stimulator spleen cells. Underlined numbers are positive values.
†Specific lysis of ^{51}Cr-labeled target cells (T) by cytotoxic attacker cells (A) was calculated from the formula % specific lysis = 100 × (experimental cpm − medium control cpm)/(maximum cpm − medium control cpm), where maximum lysis was that produced by incubation of target cells in 5% Triton X-100, and medium control was the counts for target cells incubated in medium alone. The % specific lysis at each A/T ratio was subjected to 12-point regression analysis and the figures given for cytotoxicity are taken from the regression curves, solved at an A/T ratio of 10:1. Underlined numbers are positive values from curves with a linear coefficient, r^2, between 0.80 and 1.00.

preimmunized survive for at least 30 days (8); an accelerated graft rejection time of 13–15 days is typical of females that have been preimmunized with H-Y-positive spleen cells.

When male no. 719 was 115 days old, one testis was removed and sectioned. It weighed 24 mg; the tubules were devoid of spermatogenic cells, as expected for a sex-reversed X/X male. Chromosome preparations confirmed the presence of two X chromosomes (Fig. 1), one of which carried the small additional fragment of chromatin detected in X/X Sxr mice (9). No Y chromosome was present, nor was there any other cytological indication of a translocated Y chromosome fragment. *In situ* hybridization using a Bkm-related probe showed a single focus of hybridization (Fig. 2), as expected in an X/X Sxr or Sxr′ male. This confirmed that no translocation had occurred of material from the pericentromeric region of the father's Y to an autosome.

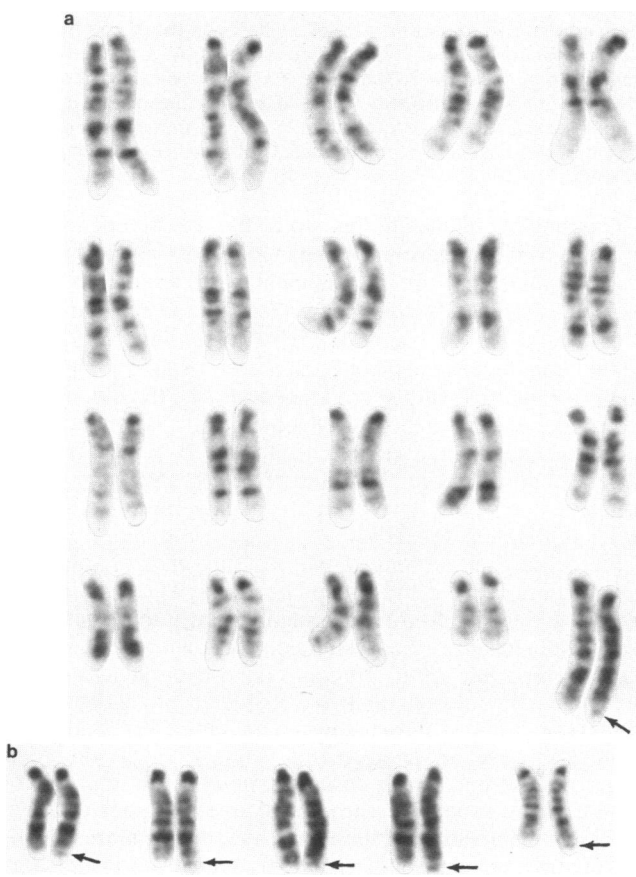

FIG. 1. (*a*) Karyotype of mouse no. 719. The extra band on one of the two X chromosomes is indicated by an arrow. (*b*) Five X chromosome pairs from mouse no. 719, each showing an extra band (arrow) on one of the X chromosomes.

The exceptional male was killed, together with one of his H-Y-negative X/X Sxr′ brothers, at the age of 300 days. Tissue samples were stored at −70°C, and DNA preparations were made. DNA fingerprinting using GATA/GACA synthetic oligonucleotide probes (7) distinguishes between the CB Y chromosome, the CB′ Y chromosome (which had a different origin; see *Materials and Methods*), the Sxr fragment, and the Sxr′ fragment (Fig. 3). The patterns are additive; thus, CB′ X/Y Sxr′ males (pattern 3′) show the CB′ Y chromosome pattern (1′) and the Sxr′ pattern (2′) superimposed, and can in this way be clearly distinguished from both X/Y(1′) and X/X Sxr′(2′) males of their own stock, as well as from CB X/X Sxr(2) and X/Y Sxr(3, i.e., 1 + 2) males (10). On DNA fingerprinting, the control male showed the 2′ pattern characteristic of X/X Sxr′ males in the CB′ stock; the exceptional male, on the other hand, showed the 1′ pattern characteristic of X/Y males in the CB′ stock (Fig. 3). This result excludes the possibility that the exceptional male could have been an X/X Sxr male from the CB stock, included in the CB′ sample in error. The fingerprinting pattern establishes the presence of the native testis-determining region, characteristic of the normal CB′ Y chromosome, and the absence of the Sxr′ region. This finding is fully consistent with the presence of H-Y antigen in the exceptional male, but it appears inconsistent with the XX karyotype and the absence of a Y chromosome.

How could the Sxr′ region have become replaced by the native testis-determining region of the Y chromosome? When an X/Y Sxr or X/Y Sxr′ male germ cell undergoes meiosis, the possibility exists that the two copies of the testis-

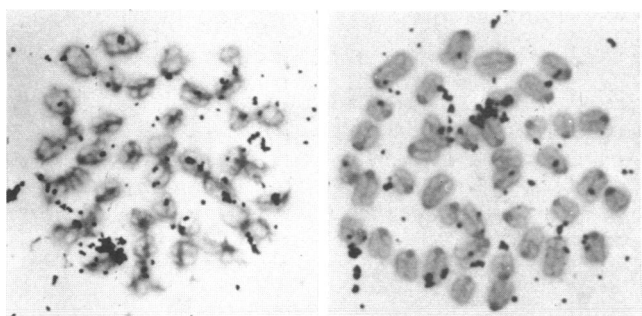

FIG. 2. *In situ* hybridization of Bkm probe to two chromosome preparations from mouse no. 719.

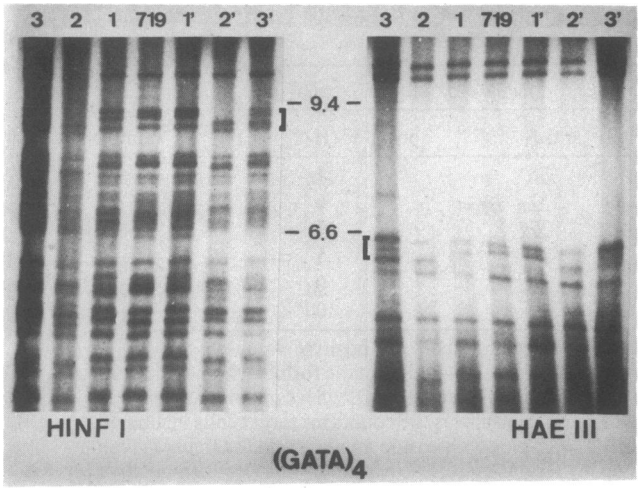

FIG. 3. DNA fingerprints of mouse no. 719 compared with X/Y(1'), X/X Sxr'(2'), and X/Y Sxr'(3')CB' strain males, also X/Y(1), X/X Sxr(2), and X/Y Sxr(3)CB strain males. Fragment lengths are indicated in kilobases. For each restriction enzyme, the region of the gel in which the polymorphic bands associated with the testis-determining region of the Y chromosome are located is indicated with a square bracket.

determining region on the Y chromosome can pair, in addition to the regular pairing of the Y chromosome with the X chromosome. Such a configuration (Fig. 4) has been reported in 14 of a total of 400 meiotic prophase figures from four X/Y Sxr males examined by electron microscopy (11). A cross-over could therefore take place between the two homologous testis-determining regions of the Y chromosome, as well as between the X and Y chromosomes. If the native testis-determining homologue were on the long arm of the Y near the centromere, the additional cross-over could produce a recombinant X chromosome bearing *Tdy* and *Hya*$^+$, but this X would be dicentric (Fig. 5*b*). Even if this dicentric avoided breakage at anaphase I (when it would form a bridge) and disjoined successfully from the accompanying X and Y chromatids at anaphase II, it would still have to inactivate one centromere if it were to continue through development. We therefore consider the successful transmission of such a dicentric to be unlikely. On the other hand, if the native testis-determining region were on the short arm of the Y (a feature of the mouse Y that is often ignored), an X chromosome carrying *Tdy* and *Hya*$^+$ could be generated without transferring a centromere from the Y (Fig. 5*d*). We conclude that *Tdy* along with *Hya* is almost certainly located on the short arm of the normal mouse Y chromosome and that our exceptional male was generated by a crossing-over event of the type illustrated in Fig. 5*d*, occurring during spermatogenesis in his X/Y Sxr' father. Since we have only observed 1 H-Y-positive male in 178 presumed X/X Sxr' males, this type of crossing-over event either must occur rarely, or it must rarely give rise to functional sperm.

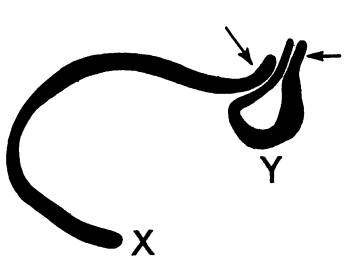

FIG. 4. Meiotic pairing configuration of X and Y Sxr chromosomes observed by electron microscopy of testis preparations from an X/Y Sxr male (modified from ref. 11).

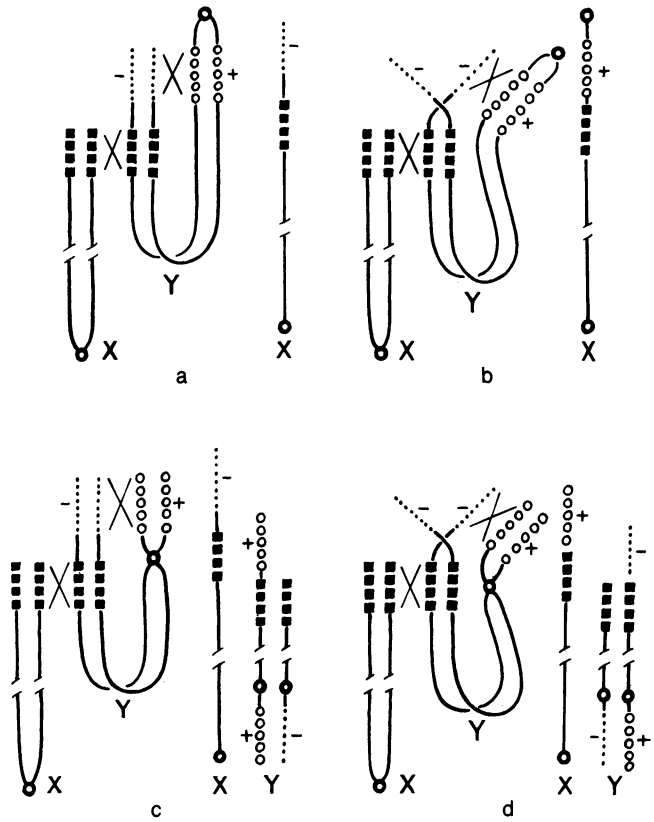

FIG. 5. Possible meiotic configurations in an X/Y Sxr' testis if pairing occurs between the two homologous testis-determining regions of the Y chromosome as well as between the X and Y chromosome. Four additional configurations are possible, with the Y chromatids not crossed; the recombinant X chromosomes generated are identical to those illustrated. In *a* and *b*, only the recombinant X chromosomes have been drawn; in *c* and *d*, the recombinant Y chromosomes have also been included. Chromosomes are not drawn to scale.

Crossing-over between the two testis-determining regions of the Y Sxr' chromosome can generate not only X but also Y chromatids that are recombinant with respect to *Hya*, including chromatids lacking *Hya* (Fig. 5 *c* and *d*), giving rise to H-Y-negative XY males. X Sxr'/O males have very small testes because, although the presence of a single X chromosome is consistent with spermatogenesis, the transition from Sxr to Sxr' has led to the loss of genetic information required for normal spermatogenesis to occur (12). H-Y-negative XY males would therefore also be expected to have very small testes and so would have been mistakenly classified as XX males in the present study.

In general, intrachromosomal crossing-over of the type illustrated in Fig. 5 *c* and *d* could occur anywhere in the Sxr region, giving rise to a recombined region rather than to a complete exchange. For example, the two Sxr and Sxr' variants reported in ref. 10 could have been generated by recombination between the two testis-determining regions of a Y Sxr or a Y Sxr' chromosome. The Sxr → Sxr' transition could also have occurred in this way, although since the father was XSxr/YSxr in chromosome constitution, an unequal cross-over between the two paired Sxr regions, leading to loss or inactivation of the *Hya* locus, seems more likely. In view of the number of repetitive sequences in the Sxr region, such events may well involve mismatching and unequal recombination. To refer to the various Sxr regions that are being generated, a new flexible terminology is required. We suggest the following: Sxr^a, Sxr (1); Sxr^b, Sxr' (3); Sxr^c, Sxr variant 4 (10); Sxr^d, Sxr' variant 4' (10); Sxr^e,

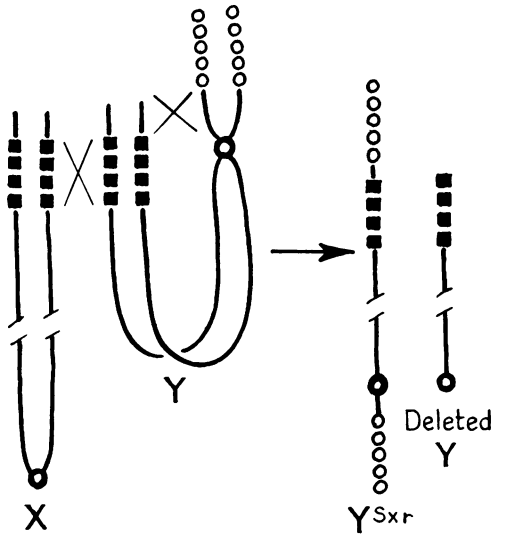

FIG. 6. Proposed origin of the original Y Sxr chromosome, from an anomalous interchromatid exchange in a normal Y.

Sxr region of mouse 719 (this paper). Sxr as a generic term would then be used to refer to any mouse Y chromosome short arm region transposed to the distal side of the X/Y pairing segment.

The short arm of the mouse Y chromosome is so short that it often escapes notice. Its existence was originally pointed out by Ford (13), and in good chromosome preparations it constitutes one of the diagnostic features that distinguishes the Y chromosome from the smallest autosome, acrocentric chromosome 19. In 1982 Evans *et al.* (9) pointed out that the Sxr body appeared cytologically similar to the short arm of the Y: the present data provide a genetic basis for this similarity. With *Tdy* and *Hya* located on the short arm of the mouse Y, the origin of the original Sxr-carrying X chromosome (1) can readily be explained as the result of a single anomalous interchromatid crossing-over event (Fig. 6), since the normal Y chromosome can occasionally be seen to loop back and associate at its tip (11). The inverted orientation of the Sxr region that is implied by the pairing configuration seen by electron microscopy (Fig. 4) would then be expected.

If these genes were located on the proximal part of the long arm, at least two anomalous events must be postulated to account for the origin of an Sxr-carrying Y chromosome. The origin of the other abnormal Y chromosome known in the mouse (14) cannot readily be explained on any simple crossing-over model, wherever *Tdy* is located.

The accompanying paper (15) shows by *in situ* hybridization that an Sxr-specific DNA probe hybridizes in the normal Y chromosome to the short arm rather than to the proximal part of the long arm. It confirms by probe hybridization that a band deleted in the Sxr → Sxr′ transition has been restored in the DNA of mouse no. 719.

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1. Cattanach, B. M., Pollard, C. E. & Hawkes, S. G. (1971) *Cytogenetics* **10**, 318–337.
2. Simpson, E., Edwards, P., Wachtel, S., McLaren, A. & Chandler, P. (1981) *Immunogenetics* **13**, 355–358.
3. McLaren, A., Simpson, E., Tomonari, K., Chandler, P. & Hogg, H. (1984) *Nature (London)* **312**, 552–555.
4. Burgoyne, P. S., Buehr, M., Koopman, P., Rossant, J. & McLaren, A. (1988) *Development* **102**, 443–450.
5. Singh, L., Phillips, C. & Jones, K. W. (1984) *Cell* **36**, 111–120.
6. Singh, L., Purdom, I. F. & Jones, K. W. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 803–815.
7. Schäfer, R., Ali, S. & Epplen, J. T. (1986) *Chromosoma* **93**, 502–510.
8. McLaren, A., Hunt, R. & Simpson, E. (1988) *Immunology* **63**, 447–449.
9. Evans, E. P., Burtenshaw, M. D. & Cattanach, B. M. (1982) *Nature (London)* **300**, 443–445.
10. Epplen, J. T., Studer, R. & McLaren, A. (1988) *Genet. Res.*, in press.
11. Chandley, A. C. & Speed, R. M. (1987) *Chromosoma* **95**, 345–349.
12. Burgoyne, P. S., Levy, E. R. & McLaren, A. (1986) *Nature (London)* **320**, 170–172.
13. Ford, C. E. (1966) *Transplantation* **4**, 333–335.
14. Eicher, E. M. & Washburn, L. L. (1986) *Annu. Rev. Genet.* **20**, 327–360.
15. Roberts, C., Weith, A., Michot, J. L., Mattei, M. G. & Bishop, C. E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6446–6449.